SH2 Domain Structures and Interactions

**Piraveen Gopalasingam, Lee Quill, Mark Jeeves
and Michael Overduin**

Abstract Src homology 2 (SH2) domains are signalling modules consisting of 100 amino acid residues which typically recognize phosphotyrosine-containing protein sequences. As such, they mediate protein–protein interactions in order to assemble signalling complexes and transduce information to alter cellular behaviour and physiology. Here we review the progress in understanding the molecular functions and structures of SH2 domains since their discovery in 1986. Diverse binding modes are revealed by studies of a variety of ligand complexes, suggesting that their mechanisms have adapted throughout the evolution in tandem with tyrosine kinases and phosphatases. Together they form the information networks responsible for development of the cellular phosphoproteome and the ensuing multicellular complexity found in higher eukaryotes. Understanding the structures, dynamics and interactions of these remarkable domains at a more predictive level is providing a rational basis for designing targeted interventions into the many disorders that result from deregulated phosphotyrosine signalling.

Keywords Phosphopeptide recognition · Phosphotyrosine binding · Protein phosphorylation · Src homology 2 domain · SH2 structure

Abbreviations

Abl	Abelson tyrosine-protein kinase 1;
APS	adaptor protein with PH and SH2 domains;
Cbl	Casitas B-lineage;
cSH2	C-terminal SH2 domain;
CSK	C-terminal Src kinase;
CTD	C-terminal domain;
ERK	extracellular signal-regulated kinase;
FERM	protein 4.1, ezrin, radixin, moesin;
GEF	guanine nucleotide exchange factor;
Grb	growth factor receptor bound protein;

P. Gopalasingam · L. Quill · M. Jeeves · M. Overduin (✉)
School of Cancer Sciences, University of Birmingham, Birmingham B15 2TT, UK
e-mail: M.Overduin@bham.ac.uk

Hck   haemopoietic cell kinase;
ITAM   immunoreceptor tyrosine-based activation motif;
Lck   lymphocyte-specific protein tyrosine kinase;
MODA   membrane optimal docking area;
NEDD   neural precursor cell expressed developmentally down-regulated protein;
NMR   nuclear magnetic resonance;
nSH2   N-terminal SH2 domain;
PDGF   platelet-derived growth factor;
PH   pleckstrin homology;
PI3K   phosphoinositide 3-kinase;
PIP2   phosphatidylinositol-4,5-bsphosphate;
PIP3   phosphatidylinositol-3,4,5-trisphosphate;
PLC   phospholipase; pSer, phosphoserine;
PTK   protein tyrosine kinase;
PTP   protein tyrosine phosphatase;
pTyr   phosphotyrosine;
SAP   SLAM-associated protein;
SH2   Src homology 2;
SH3   Src homology 3;
Shp2   Src homology 2-containing phosphotyrosine phosphatase;
SLAP   Src-like adaptor protein;
SOCS   suppressor of cytokine signalling;
STAT   Signal Transducer and Activator of Transcription;
Syk   spleen tyrosine kinase;
Zap70   zeta chain-associated protein kinase of 70 kDa

1 Introduction to SH2 Domains

1.1 Biological Functions of SH2 Domains

Tyrosine phosphorylation contributes to only approximately 0.5 % of the total phosphoproteome, yet it plays critical roles in eukaryotic cell regulation (Cohen 2002). The enzymes which control tyrosine phosphorylation require careful spatiotemporal regulation in order to potentiate cellular responses to specific extracellular stimuli. The most crucial regulatory module is the *Src* Homology 2 (SH2) domain, which was originally identified in viral oncogenes. Tony Pawson's group first discovered the SH2 domain as a non-catalytic region of the *v-fps/fes* gene which is required for tumour transformation (Sadowski et al. 1986). Subsequent sequence alignments mapped SH2-like regions within a diverse array of intracellular signalling proteins found in eukaryotes. Since then, the importance of this module in signalling has been recognised as being central for regulating cell

growth, differentiation and migration, as well as mediating complex signalling pathways such as in the immune systems (Liu and Nash 2012). Tyrosine phosphorylation is cell-type specific as well as time dependent, and hence has provided a foundation for the development of diverse tissues, organs and organisms. These processes are fundamentally based on the differential expression and regulated activities of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs) which often contain SH2 domains (Yaffe 2002).

In order to organize responses to diverse signals, elaborate networks of divergent SH2 domain-containing proteins have evolved that accurately detect and transform molecular events into appropriate cellular responses. SH2 proteins are widely considered to be the major readers of the tyrosine phosphoproteome and form the cell's primary means of phosphotyrosine (pTyr) recognition. The roles of SH2 domains within tyrosine phosphorylation-dependent pathways generally involve localizing the PTK and PTP enzymes to specific substrates and signalling effectors, as shown for the well-characterized Ras-MAPK, JAK-STAT and MEK/ERK pathways. The SH2 domain-containing proteins within these signalling cascades display modular architectures, with various other structural domains, such as pleckstrin homology (PH), PTB, PDZ, SH3 and WW domains, mediating interactions with proteins and membrane surfaces, as well as catalytic domains such as GTPases, inositol phosphatases and phospholipases. There are also SH2 proteins that do not possess enzymatic activity and instead act purely as adaptor proteins that link other proteins together. Prominent examples of such signalling adaptors include the Gab1, Grb2, SOS, SOCS and VAV1 proteins. Finally some SH2 proteins possess enzymatic functions but can also serve separately as adaptors, with distinct consequences in downstream signalling if either of these functions is disrupted. One such example is the PTP Shp2, where Noonan and LEOPARD syndromes arise from mutations that compromise its adaptor SH2 and catalytic domains (Gelb and Tartaglia 2006).

The primary biochemical function of the SH2 domain is to selectively recognize polypeptides containing a phosphotyrosine (pTyr) along with specific contiguous residues. As a small, stable structural unit (Fig. 1) that is generally flexibly tethered to a larger protein, the SH2 domain is able to diffuse through local intracellular spaces until a protein ligand is bound. The reversible interaction between SH2 domains and pTyr motifs thus provides regulated co-localization of multiple proteins within a signalling cascade, allowing further enzymatic modifications and propagation of signalling cascades. There are thousands of pTyr sites in the human proteome (Sharma et al. 2014) which mediate interactions with cognate binding partners in hundreds of interconnected pathways that each mount appropriate responses to changes in diverse cellular microenvironments (Hunter 2009). It is the SH2 domain that provides order to this signalling complexity, and its functional integrity is of paramount importance to the cell.

Extracellular signals are conveyed into the cell through growth factor receptors which generate intracellular tyrosine phosphorylation sites. These processes have been extensively studied in the cases of the cell surface receptors for insulin and various growth factors such as epidermal growth factor (EGF) and platelet-derived

Fig. 1 Structure of a representative SH2 domain structure with the helices, strands and loops labelled
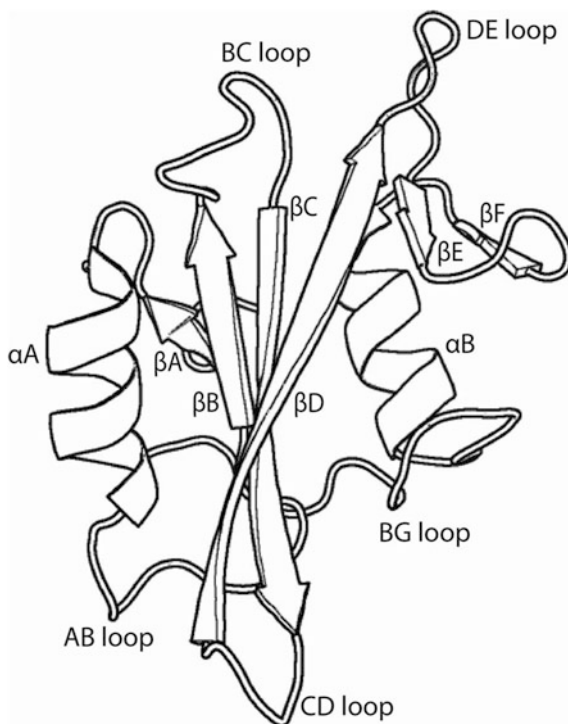
growth factor (PDGF). These transmembrane receptors multimerise and the activated cytoplasmic kinase domains phosphorylate tyrosine residues in the C-terminal tails of the other monomer. These pTyr sites serve as docking sites for various SH2 domains that act to potentiate the initial signal, with other post-translational modifications including serine and threonine phosphorylation, ubiquitination as well as GTP hydrolysis also coming into play (Deribe et al. 2010).

Some SH2 domains are known to directly regulate enzyme activity. In the PTKs Src, Fyn, Lyn and Yes the SH2 domain binds a phosphopeptide sequence near the C-terminus, thus occluding the kinase active site and autoinhibiting catalytic activity. In contrast, the Abl kinase can either be autoinhibited or activated by its SH2 domain, depending on its conformational state (Hantschel et al. 2003; Nagar et al. 2006; Filippakopoulos et al. 2009). The PTPs Shp1 and Shp2 contain two SH2 domains N-terminal to the catalytic domain, the first of these forms extensive contacts close to and occluding the PTP active site, thus stabilizing an inactive conformation (Hof et al. 1998). The PTP is activated through stable interaction of its tandem SH2 domains with pairs of pTyr motifs in cell surface receptors. Thus SH2 domains not only serve to connect the various components of signalling pathways in *trans* interactions but act in *cis* to modulate enzymatic function.

Depending on the signalling context of the SH2 domain-containing protein, a pTyr interaction may require specific residence times. Growth and differentiation

pathways require tight control over signal generation as even minor changes could lead to detrimental consequences. Residence times can be modulated by varying the affinity and specificity of a binary peptide:SH2 interaction, serving to fine-tune transient complexes in a cascade. Although SH2 domains are singularly important for the fidelity of intracellular signal transduction, other interaction domains within their host proteins and their homologs and complexes can compensate for disruption or loss. Such redundancy in a pathway is commonly observed in complex multicellular organisms and confers slack in the system (Kaneko et al. 2012b).

Although the fold of SH2 domains is highly structurally conserved, divergent features can endow them with specialized functions. For example, the adaptor protein Crk contains an atypical SH2 domain with a proline-rich loop (Fig. 2) that is recognized by the SH3 domain of Abl (Donaldson et al. 2002) in a phosphorylation-dependent manner (Anafi et al. 1996). The SH2 domain of the adaptor protein SOCS3 contains an unstructured 35 residue PEST motif sequence which is enriched in proline, glutamic acid, serine and threonine residues (Babon et al. 2006).

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          βA    AA         αA              AB    βB       BC        βC          CD
Vav1     WYAGPME-----RAGAESILAN------RSDGTFLVRQRVKDAAEFAISIKYNV-----E   659
p85β-C   WYVGKIN-----RTQAEEMLSG-----KRDGTFLIRES-SQRGCYACSVVVDG-----D    664
Grb2     WFFGKIP-----RAKAEEMLSKQ-----RHDGAFLIRESESAPGDFSLSVKFGN-----D   104
SLAP2    WLYEGLS-----REKAEELLLLP----GNPGAFLIRESQTRRGSYSLSVRLSRPASWDR     144
Src      WYFGKIT-----RRESERLLLNA----ENPRGTFLVRESETTKGAYCLSVSDFDNAKGLN   201
Crk      WYWGRLS-----RQEAVALLQG------QRHGVFLVRDSSTSPGDYVLSVSENS-----R    56
Grb7     WYHGRIS-----REESQRLIGQQ----GLVDGLFLVRESGRNPQGFVLSLCHLQ-----K   476
Abl1     WYHGPVS-----RNAAEYLLSS-----GINGSFLVRESESSPGQRSISLRYEG-----R    170
Shp2-N   WFHPNIT-----GVEAENLLLTR-----GVDGSFLARPSKSNPGDFTLSVRRNG-----A    50
SAP      WYHGKIS-----RETGEKLLLAT-----GLDGSYLLRDSESVPGVYCLCVLYHG-----Y    50
PLCγ1-N  WFHGKLGAGRDGRHIAERLLTEYCIETGAPDGSFLVRESETFVGDYTLSFWRNG-----K   604
APS      WFHGTLS-----RVKAAQLVLAG---GPRNHGLFVIRQSETRPGEYVLTFNFQG-----K   463
STAT1    WNDGCIMGF--ISKERERALLKD-----QQPGTFLLRFSESSR-EGAITFTWVE-----R   619
Tyk2     GIHGPLL-----EPFVQAKLR-------PEDGLYLIHWSTSHPYRLILTVAQRSQA-PDG   497
Spt6     YIKRVIAHPSFHNINFKQAEKMM---ETMDQGDVIIRPSSKGENHLTVTWKVSDG----I  1377
Cbl      GYMAFLT-----YDEVKARLQKF----IHKPGSYIFRLSCTRLGQWAIGYVTADG----N   313

          βD   βD'    DE      βE     EF      βF  FB      αB              BG   βG
Vav1     VKHIKIMTAE-----GLYRITEK----KAFRGLTELVEFYQQNSLKDC-FKSLDTTLQFP    709
p85β-C   TKHCVIYRTA----TGFGFAEPY----NLYGSLKELVLHYQHASLVQHN-DALTVTLAHP    715
Grb2     VQHFKVLRDG-----AGKYFLWVV-----KFNSLNELVDYHRSTSVS----RNQQFLRDI    151
SLAP2    IRHYRIHCLD----NGWLYISPR----LTFPSLQALVDHYSELAD------DICCLLKEP    190
Src      VKHYKIRKLD----SGGFYITSR----TQFNSLQQLVAYYSKHAD------GLCHRLTTV    247
Crk      VSHYIINSSGXGVSPSRLRIGD-----QEFDSLPALLEFYKIHYL------DTTTLIEPV    118
Grb7     VKHYLILPSEE---EGRLYFSMDD-GQTRFTDLLQLVEFHQLNRG------ILPCLLRHC    526
Abl1     VYHYRINTAS----DGKLYVSSE----SRFNTLAELVHHHSTVAD------GLITTLHYP    216
Shp2-N   VTHIKIQNTG-----DYYDLYGGE----KFATLAELVQYYMEHHGQLKEKNGDVIELKYP    101
SAP      IYTYRVSQTE---TGGSWSAETAPGVHKRYFRKIKNLISAFQKPDQ-----GIVIPLQYP    101
PLCγ1-N  VQHCRIHSRQDA-GTPKFFLTDN----LVFDSLYDLITHYQQVPLRCN---EFEMRLSEP    656
APS      AKHLRLSLNG-----HGQ-----CHVQHLWFQSVLDMLRHFHTHPIPLESGGSADITLRSY   514
STAT1    SQNGGEPDFHAV--EPYT------KKELSAVTFPDIIRNYKVMAAEN-IPENPLKYL-YP    669
Tyk2     MQSLRLRKFPIEQQDGAFVLEGWG---RSFPSVRELGAALQGCLLR---AGDDCFSLRRC    550
Spt6     YQHVDVREEGKENAFSLGATLWINS--EEFEDLDEIVARYVQPMAS-----FARDLLNHK   1430
Cbl      ILQTIPH--------------------NKPLFQALIDGFRE----------GFYL-FP     340
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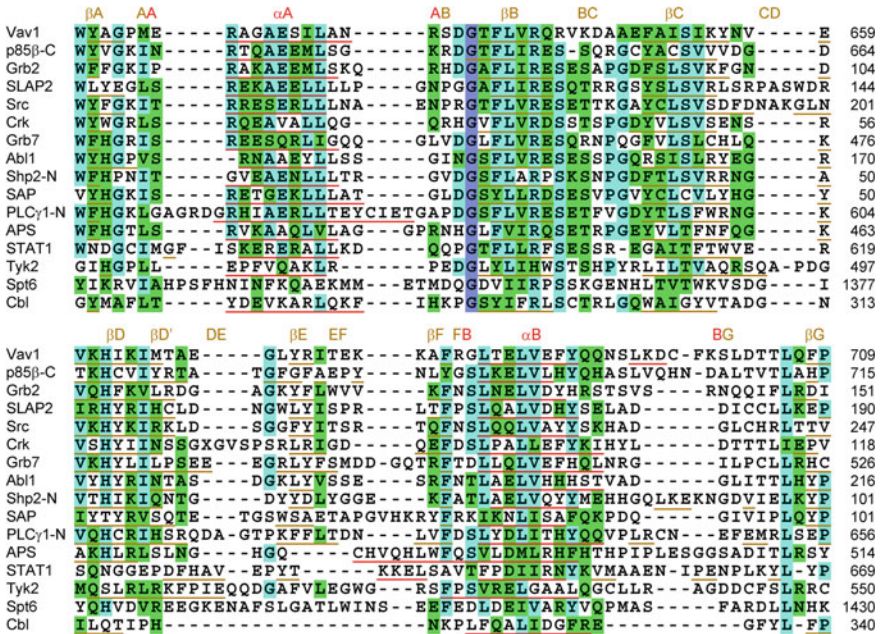
Fig. 2 Alignment of representative SH2 domain sequences showing secondary structure elements. Alignment of human SH2 domain sequences with secondary structures from Vav1 (2mc1), PI3K p85 β subunit (2y3a), Grb2 (3wa4), SLAP2 (4m4z), Src (1fmk), Crk (1ju5), Grb7 (2qms), Abl1 (1opk), Shp2 (3tkz), SAP (1d1z), PLCg1 (4fbn), APS (1rpy), STAT1 (1yvl), Tyk2 (4po6), Spt6, Cbl (3pfv), with "N" and "C" indicating the N or C terminal domain of a tandem SH2 proteins. Aligned with ClustalW using the BLOSUM weightings. Helices and strands are coloured *red* and *gold*, respectively, and labelled along with the intervening loops. Fully conserved, identical, and similar residues are highlighted in *blue*, *aqua* and *green*, respectively. A proline rich sequence in the Crk DE loop is indicated by an "X"

This PEST sequence undergoes phosphorylation as a means of modulating the half-life of the SOCS protein, thus providing a regulatory mechanism within cytokine/JAK-STAT signalling that utilises the ubiquitin-mediated degradation pathway (Yoshimura et al. 2007). Thus SH2 domains can both bind and be bound by partner proteins, and also expose unique surface features for regulating protein activity and degradation. As such, SH2 domains are multifaceted contributors to pTyr signalling systems and thus have been described as highly regulated readers of the pTyr signals that are produced and deleted by PTK and PTP enzymes respectively (Lim and Pawson 2010).

1.2 Evolution and Breadth of the SH2 Superfamily

Tyrosine phosphorylation first evolved as an intracellular signal in early metazoans (Liu and Nash 2012). The number and complexity of pTyr-based systems diversified with increasing organismal complexity (Liu et al. 2011). The expansion of the number of genes encoding SH2 domains during this period paralleled the evolution of divergent PTKs. The transcriptional regulator Spt6, which contains two SH2 domains—one canonical and the other uniquely folded and phosphoserine-binding, could be the progenitor of the superfamily, although another contender is the transcription activating STAT protein (Liu and Nash 2012).

Genome sequencing of the unicellular choanoflagellates has identified a large number of pTyr signalling proteins (Manning et al. 2008). Cross-species genome comparisons suggest that pTyr signalling networks emerged in these organisms, which are representative of the ancestral animal evolution. Gene duplication led to rapid growth of the number of SH2 domain-containing proteins, with domain gain and loss and mutational events leading to the development of novel functions and tissue-specific regulation whilst maintaining the basic conserved signalling network (Liu and Nash 2012).

To date 111 genes, encoding 121 SH2 domains, have been identified in humans (Liu et al. 2012). Those proteins containing pairs of SH2 domains include SYK, Zap70, Shp1, Shp2, the phospholipase C homologs PLCγ1 and PLCγ2, as well as the p85α and p85β regulatory subunits of phosphoinositide-3 kinase (PI3 K, Fig. 2). The presence of two SH2 domains in a protein confers additional affinity and specificity by imposing spatial constraints to a binary interaction (Eck et al. 1996), while single SH2 domains generally provide transient interactions with specific ligands.

1.3 Biomedical Significance of SH2 Proteins

At least 23 SH2 domain-containing proteins have been implicated in disease causation to date. This represents around 1 in every 5 members of the human SH2 superfamily, and effects chronic conditions such as diabetes, Noonan and

LEOPARD syndromes and immune system disorders. This has stimulated numerous efforts to design SH2-specific inhibitors, as reviewed recently (Kraskouskaya et al. 2013), although the most successful route to intervention to date has been from the design of competitive inhibitors of the kinase domains of these proteins.

Mutations within SH2 domains underpin the molecular pathogenesis of various diseases, thus understanding their mechanism of dysregulation is vital for designing clinical interventions. Functional effects of SH2 mutations range from enzyme hyperactivation to changes in pTyr:SH2 affinity and specificity. For the latter, a spectrum of deleterious mutations have been documented wherein pTyr:SH2 binding is completely abolished and in other cases altering either the residence times of phosphopeptide in the SH2 pTyr-pocket or their ligand specificity. Indeed it has been demonstrated that a single point mutation in the Src SH2 domain alters its peptide specificity to resemble the Grb2 SH2 domain (Kimber et al. 2000).

Phosphotyrosine-based signalling is widely utilised in growth factor-mediated cascades so it is unsurprising that mutations affecting these pathways are implicated in cancer initiation, progression and metastasis. A subset of these mutations lie within SH2 domains. The Shp2 protein was the first oncogenic PTP discovered, with germline mutations in its SH2 domains increasing the likelihood of hematological malignancies including juvenile myelomonocytic leukaemia. Germline and somatic mutations of Shp2 have also been implicated in cases of colon, skin, thyroid and brain cancer (Chan et al. 2008). Similarly, mutations in the C-terminal SH2 domain of the RASA1 protein which regulates the GTPase activity of Ras are found in Basal-cell carcinoma (Friedman 1995). A mutation in the first SH2 domain of PI3K's regulatory p85 subunit compromises the lipid kinase activity and may contribute to insulin resistance (Baynes et al. 2000), while a deletion near the second SH2 domain contributes to the development of solid tumours (Philp et al. 2001).

Several immune system disorders are caused by SH2 domain alterations. The X-linked Lymphoproliferative (XLP) syndrome is brought about by mutations in the *SH2D1A* gene encoding SLAM-associated Protein (SAP) and affects males only. Missense mutations localise to the SLAM-peptide binding site, suggesting abrogation of peptide binding to SAP drives pathogenesis of XLP by dysregulating pathways involved in adaptive immune system activation (Poy et al. 1999). Mutations in the Bruton's tyrosine kinase (BTK) gene including in its SH2 domain reduce the number of mature B-cells and thus cause a hereditary immunodeficiency known as X-linked agammaglobulinemia (Valiaho et al. 2006). Mutations in the Zap70 gene including in its SH2 domain can destabilise the protein, thus reducing T cell numbers and leading to a severe immunodeficiency (Cauwe et al. 2014).

Increased vulnerability to infection can also be caused by mutated SH2 domains. Dominant negative mutations in the SH2 domain of STAT1 alter the protein's phosphorylation and DNA-binding activity. This impairs the interferon and interleukin responsiveness of the cell and leads to susceptibility to mycobacterial infection (Tsumura et al. 2012). A mutation of the SH2 domain of STAT5B compromises cellular sensitivity to growth hormone, with the resulting immunodeficiency leading to growth failure, recurrent bacterial and viral infections (Kofoed et al. 2003).

2 SH2 Domain Structure and Function

2.1 SH2 Domain Fold

The fold of the SH2 domain is optimized for reading the pTyr signals which are dynamically created in higher eukaryotes and tuning the ensuing cellular responses. The three dimensional structures of ∼70 SH2 domains have been determined. They reveal a highly conserved topology comprising a β sheet consisting of seven anti-parallel strands (βA-βG) that are flanked at either end by a conserved pair of helices (αA and αB) (Waksman et al. 1992, 1993). The common nomenclature specifies the positions of residues relative to these secondary structure elements (Fig. 1), with βB5 being the fifth residue in the conserved second strand. Comparison of the SH2 domain structures in both free and phosphopeptide-bound states has collectively identified the architecturally conserved N-terminal region as the mediator of pTyr binding. This is in contrast to the C-terminal segment of the SH2 domain which exhibits much broader structural variability, owing to its specificity for hydrophobic residues that are found in the various peptide ligands and protein partners.

2.2 Phosphotyrosine Recognition by SH2 Domains

The elucidation of the key structural features governing SH2 recognition of pTyr containing sequences has not only provided key information on how pTyr residues are read, but also offers potential insights into the use of SH2 domains as diagnostic tools and as druggable targets for the design of therapeutic agents for an array of human diseases (Sawyer 1998).

Resolving the structural basis of pTyr recognition has been a critical step to informing our understanding of SH2 function. Accessing such information has relied on the utility of X-ray crystallography to illuminate the binding interfaces between signalling complexes at high resolution. Crystal structures for the majority of SH2-ligand complexes reveal that the pTyr-peptide adopts an extended conformation and binds perpendicular to the central β-strands of the SH2 domain. In almost all cases, the pTyr substrate is accommodated in a moderately deep binding pocket, with the phosphate oxygens participating in an extensive network of hydrogen bonds and electrostatic interactions that act to stabilise the complex. Conserved arginine residues Arg αA2 and Arg βB5 have been identified as key residues and mediators of phosphate group binding. The phenyl moiety of pTyr stabilises the interaction through the formation of an amino-aromatic bond to a conserved Arg within the FLVR motif (Fig. 2) in conjunction with additional interactions involving side chain and main chain groups from residues in the βD strand (Waksman et al. 1992; Waksman et al. 1993; Waksman and Kuriyan 2004). The second binding pocket within the SH2 domain provides hydrophobic

interactions for engaging residues positioned C-terminal to the pTyr and confers specificity for ligand interactions (Liu et al. 2012).

A central paradigm in SH2-dependent signalling asserts that the lifetime of global tyrosine phosphorylation is precisely controlled by the lifetime of the individual SH2-containing complexes that make up the signalling network. These signalling complexes function collectively as part of these networks to drive the propagation of phosphorylation cascades leading to the activation of a particular cellular response. The rate of decay of SH2-dependent interactions in any given network is therefore crucial to the maintenance of the signal and the downstream activation of diverse cellular processes. Several studies have reported the dissociation constant for the binding interaction of SH2 domains with pTyr ligands to have affinities in the range of 0.1–10 µM (Ladbury and Arold 2011; Jones et al. 2006). Despite the high specificity of these canonical interactions the moderate affinities reported are crucial to the transient association and dissociation rates necessary for complex formation, and for the subsequent control of cellular signalling cascades. SH2 domains artificially engineered to possess stronger binding (known as pTyr superbinders) of pTyr-containing sequences have detrimental consequences for signal transduction, illustrating the importance of maintaining the critical balance between binding affinities of signalling and adaptor proteins and the propagation of signals (Kaneko et al. 2012a).

The highly conserved pocket responsible for recognition of pTyr has been estimated to contribute approximately half of the energy associated with ligand binding (Waksman et al. 2004), while the comparatively variable hydrophobic pocket found in the C-terminal region of the SH2 domain provides essential specificity for hydrophobic residues on cognate ligands. The large energetic contribution of the pTyr residue to SH2-domain binding provides an efficient basis for discriminating between phosphorylated and non-phosphorylated sequences, and therefore provides a mechanism for the rapid termination of cell signalling events regulated by tyrosine phosphorylation. Additional structural features of SH2 domains also serve as crucial determinants for pTyr recognition, for example, distinct conformational states of the EF and BG loops are important for restricting ligand access and influence the specificity of SH2 domains for the residues occupying the second, third or fourth position that lies C-terminal to the pTyr residue (Liu et al. 2006, 2011).

2.3 *Selectivity for Primary Sequence Motifs*

The first SH2 structure solved in the presence of a pTyr ligand was that of the Src SH2 domain complexed with a high affinity ligand peptide derived from the middle T-antigen. The structure revealed the precise architecture of the pTyr binding pocket and illuminated both the hydrophobic pocket coordinating the +3 Ile (Waksman et al. 1993) and the canonical pY-E-E-I peptide recognition sequence (Fig. 3a). The SH2 domain structures that appeared subsequently exhibited highly conserved pTyr binding pockets, and showed notable diversity in their binding
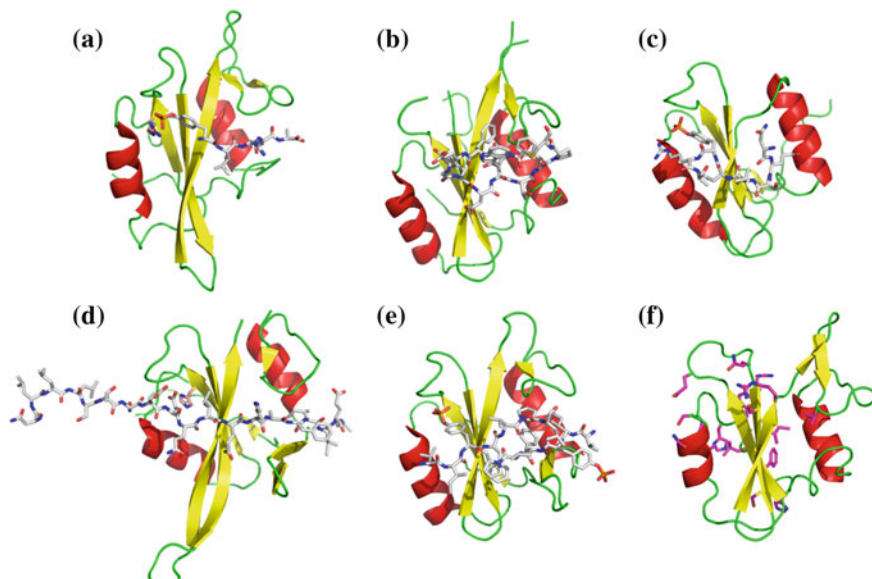
Fig. 3 Diversity of SH2 domain binding mechanisms. SH2 domains are shown as backbone ribbons and the bound peptide ligands are displayed using stick representations. **a** The classical orientation of the Src SH2 domain binding pY peptide is shown, with the key arginine residue which coordinates the phosphate of the pTyr shown in stick representation. **b** The Grb7 SH2 domain bound to a cyclical non-phosphorylated tyrosine peptide. **c** The Cbl SH2 domain bound to the phosphorylated Met receptor tail peptide in a reverse orientation. **d** The Tyk SH2 domain bound to a peptide with a glutamate in the usual pY position. **e** The Shp2 N-terminal SH2 domain complexed with two pTyr-containing peptides. **f** The Abl SH2 domain with residues that undergo chemical shift changes upon binding of PtdIns(4,5)P$_2$ shown in stick representation

modes for neighbouring ligand residues. For example, the Grb2 SH2 domain has been identified to have a strong propensity for a +2 Asn (pY-x-N-x motif) as a result of a Trp residue in the DE loop of the SH2 domain. Other SH2 structures have revealed key interactions extending beyond the +3 position. A combination of peptide array and structural data elucidated for the BRDG1 SH2 domain demonstrated a strong preference for a hydrophobic Leu residue at the +4 position. Additionally, SH2 domains belonging to PLC-γ1 have been shown to preferentially recognise longer more extended peptides with contacts forming up to the +5 position (Kaneko et al. 2010; Huang et al. 2008).

2.4 Specificity for Ligand Secondary Structures

Conformational Adaptability: Ligands for SH2 domains are typically found in unstructured sequences of cytoplasmic proteins. For example, the PLC-γ1 C-terminal SH2 domain binds disordered phosphopeptides derived from the PDGF

receptor. On contact, the mobility around the pTyr and +3 residues becomes restricted (Finerty et al. 2005). The bound peptide ligands typically assume an extended conformation that stretches between the pTyr and +3 pockets and lies across strand βD (Waksman et al. 1993). In the case of the Src SH2 complex (mentioned in Sect. 2.3), the four residues that are predominantly recognized are the pTyr and lle(+3) residues, which are buried, and the intervening Glu(+l) and Glu (+2) residues, which are largely solvent exposed and form complementary electrostatic interactions with positively charged residues on the SH2 surface. The binding conformations of such phosphopeptide ligands can be either extended or helical depending on the presence of an Ile or Leu at the +3 position, due to their respective intrinsic structural propensies (Nachman et al. 2010). In contrast, an alternative β-turn conformation is induced in Src ligands having an Asn at the +2 position, which can supplant the hydrophobic residue at the +3 position as a key determinant of SH2 binding specificity. Hence the Src SH2 domain can accommodate bound phosphopeptides having either extended, helical or β-turn conformations depending on their sequence context, indicating the plasticity of ligand recognition. Moreover, these various binding modes may be differentially regulated by phosphorylation, for example of Src residue Tyr213 within the EF loop of its SH2 domain by the PDGF receptor (Stover et al. 1996).

Ligands Forming Turns: In contrast to most SH2 ligands, a β-turn is the default binding conformation of peptides bound by the growth factor receptor bound protein Grb2, which serves as an adaptor that links receptors at the plasma membrane with cytoplasmic kinases. Its SH2 domain binds peptides containing the pY-V-N-V sequence in a type 1 β turn conformation, providing a basis for the design of selective inhibitors (Rahuel et al. 1996). The Asn residue at the pTyr +2 position forms hydrogen bonds with the protein backbone and the peptide itself, as well as contacting the tryptophan which is the first residue of the loop between βE and βF. One of Grb2's partners is the CD28 receptor on the T-cell surface, which offers a pY-M-N-M sequence as a ligand. The binding mode is that of a twisted U-shaped turn that deviates from a canonical type-I β-turn due to alternative packing of the +1 and +2 residues (Higo et al. 2013). In contrast, amyloid precursor protein-derived peptides bind to the Grb2 SH2 domain but have a proline residue at the pTyr+3 position that precludes formation of a β-turn and rather supports flipping of helix-turn-helix conformation (Das et al. 2011).

Thus, rather than each SH2 domain having a single preferred binding mode, each interaction must balance both the intrinsic structural propensity of the ligand motif as well as specific contacts with the SH2 domain at each position, particularly between the pTyr and +3 position. This indicates the adaptability of SH2 domains that allows them to recognize diverse ligands depending on their local sequence and conformational fit, both of which play significant roles in generating productive signalling complexes. It can be inferred that such complexes are not evolved for maximum affinity of a single ligand, but rather to allow reversible and specific binding profiles for arrays of potential biological partners.

2.5   Noncanonical Ligands of SH2 Domains

Phosphoserine Specificity: Although pTyr-containing peptide motifs are clearly the dominant ligands of most SH2 domains, there are well-documented exceptions. In fact, the earliest SH2 domain may have been a phosphoserine peptide binding module. The only SH2 domains in yeast are found in the transcription factor Spt6 (Lim and Pawson 2010). This protein is conserved in plants and slime moulds, and is known to bind to the C-terminal domain (CTD) of RNA polymerase II (Yoh et al. 2007). The CTD contains phosphorylated YSPTSPS repeats that are recognized by the intertwined pair of SH2 domains of Spt6 (Sun et al. 2010; Diebold et al. 2010). Only the N-terminal SH2 domain of the tandem has a functional phosphopeptide binding pocket, which includes the invariant arginine found in eukaryotic SH2 domains. In contrast, the C-terminal SH2 domain lacks this canonical site and instead contacts the CTD through a positively charged area on its surface. Thus Spt6 SH2 domains may represent progenitors of the pTyr-binding SH2 domains that emerged in parallel with protein tyrosine kinases during early eukaryotic evolution.

Backwards Binding: The divergent SH2 domain of the Cbl ubiquitin ligase binds pTyr peptides with inverted specificity. The Cbl protein structure possesses three closely interacting N-terminal domains: a four-helix bundle, a calcium-binding EF-hand and a divergent SH2 domain (Fig. 3c). Its SH2 domain is structurally unusual in that the neighbouring four-helix bundle completes the pTyr pocket, and strands D', E and F are missing as is the G loop (Meng et al. 1999). The ligand specificity of Cbl SH2 domain is atypical in that it prefers ligand sequences having an Asn, Aspartate or Arg residue immediately preceding the pTyr. Moreover, through interactions with the four-helix bundle this selectivity extends six residues N-terminal to the pTyr in the case of the adaptor protein APS (Hu and Hubbard 2005). As a further twist, the Cbl SH2 domain accommodates the Met receptor's ligand sequence in the reverse orientation, such that the residues N-terminal to the pTyr extend across the β sheet to occupy the positions where the C-terminal specificity determinant usually reside (Ng et al. 2008). This feature provides c-Cbl with a unique cohort of partners that are unavailable to other SH2 proteins, and allows ligands to bind in potentially two opposite orientations.

Phosphopeptide Independent Binding: Over time some divergent SH2 domains have lost the ability to recognize pTyr ligands. Those of the Rin2 and Tyk2 proteins contain a histidine rather than the conserved arginine in the βB5 position which is typically essential for pTyr binding, while SH2D5 contains a tryptophan here. A recent Tyk2 structure reveals that its tandem FERM and SH2 domains form an extensive interface with each other and contact adjacent box motifs in the interferon-α receptor tail (Wallweber et al. 2014). This unusual SH2 domain employs what is normally the pTyr binding pocket to instead recognize a glutamate residue in the receptor tail, while a hydrophobic groove formed by the βG1 strand and EF loop contacts extended aliphatic residues between five and seven residues C-terminal to this pTyr mimicking residue (Fig. 3d). Thus pTyr

ligands can over time be replaced by a glutamate to support constitutive, non-phosphorylation dependent interactions.

Another unique case involves the SH2 domain of the SLAM-associated protein (SAP), which binds a SLAM receptor sequence even when it is not phosphorylated on the target tyrosine residue. The affinity of the unphosphorylated ligand is four to five-fold lower than the pTyr-containing form, and involves recognition of residues at the +2 and -3 positions, leading to a three-pronged fit (Morra et al. 2001; Poy et al. 1999; Sayos et al. 1998). The preference for residues N-terminal to the tyrosine results from a parallel β sheet interaction with strand D as well as hydrogen bonding contacts with a glutamate at position αA6. The unphosphorylated tyrosine residue occupies the pTyr pocket along with ordered water molecules instead of the normal phosphate group. Thus although a phosphate group is usually required on a tyrosine ligand, some SH2 domain interactions with established partners are sufficiently robust that this dependency is no longer absolute. A further wrinkle is offered by SAP signalling. Its SH2 domain contains a loop that is recognized by the Fyn SH3 domain, thus allowing assembly of a ternary complex with the SLAM receptor. The structure of this signalling complex reveals that strand F and helix B of the SAP SH2 domain bind the Fyn SH3 domain through a non-canonical interaction. This interaction would preclude the Fyn's auto-inhibited conformation, thus promoting kinase activation (Chan et al. 2003). The SH2 domain remains free to bind a peptide from the SLAM receptor in its standard 3-pronged mode, thus illustrating SAP's ability to act as a multifaceted adaptor protein.

Grb7 belongs to a subfamily of SH2-containing adapters that includes Grb2, and interacts with both phosphorylated and unphosphorylated sequences in activated upstream partners (Pero et al. 2002). The structure of its SH2 domain bound to an unphosphorylated cyclic peptide reveals that the Tyr-Asp-Asn motif is positioned similarly to the canonical pTyr-containing ligands of its relative Grb2 (Ambaye et al. 2011). Despite the absence of a phosphate group, the tyrosine side chain of the ligand fits into the pTyr binding pocket, while the neighbouring residues make contacts that stabilize the bound pseudo-β-turn of the peptide and pack over the EF loop (Fig. 3b). The cyclic peptide binds specifically to Grb7 with an affinity of 35 μM despite the lack of a pTyr, providing a basis for the development of selective cell-permeable inhibitors for this putative cancer target.

Ligands that Double Up: While most peptide ligands bind as monomers to a SH2 domain, a unique class of peptides can bind as dimers. A pair of pY-F-V-P sequence containing peptides were co-crystallized with the N-terminal SH2 (nSH2) domain of Shp2 (Zhang et al. 2011b). They form an antiparallel β-sheet when bound such that one peptide's pTyr fits canonically while the other bound peptide extends across the central β sheet of the domain (Fig. 3e). Such dual peptide interactions with a single SH2 domain could conceivably serve as a scaffold for two ligand molecules by supporting their dimerization.

Lipid Interactions: Several studies have reported lipid binding activities within SH2 domains. The interactions of phosphatidylinositol-3,4,5-trisphosphate (PIP$_3$) with the SH2 domains of PI3K, Src, Abl and Lck were discovered using radio-labelled vesicle binding experiments (Rameh et al. 1995). Lipid binding is

competed with phosphopeptide binding, indicating overlapping sites. The lipid-specific interaction was confirmed by assays including PIP_3-bead binding (Wang et al. 2000), radioligand binding, fluorescence titration, and gel filtration (Ching et al. 2001), and indicated an affinity in the lower μM range as well as a requirement for long chain lipid. This interaction was contested by an NMR study that showed lack of specificity for soluble phosphoinositides by the p85 cSH2 domain (Surdo et al. 1999), although a PIP_3 molecule was shown to bind weakly to the pTyr site. In a similar vein, the C-terminal SH2 domain of PLCγ binds PIP_3 with an affinity of 2.4 μM and in a manner that competes with the activated PDGF receptor (Rameh et al. 1998). PIP_3 binding to both the SH2 and PH domains of this protein suggests concerted stimulation of the catalytic activity of PLCγ, thus leading to inositol trisphosphate (IP_3) generation and intracellular calcium release (Bae et al. 1998). The Abl SH2 interaction with phosphatidylinositol-4,5-bisphosphate (PIP_2) was validated by NMR and vesicle binding experiments (Tokonzaba et al. 2006). The His-Tyr-Arg sequence on βD is crucial based on the NMR chemical shift and mutagenesis data (Fig. 3f). Hence various SH2 domains which are found in membrane-localized proteins appear to have acquired PI lipid binding capabilities that depend on electrostatic attraction within an area around the pTyr pocket. Although generally weak and of limited stereospecificity, such lipid interactions may orient signalling domains on membranes, particularly in the cases of myristoylated kinases and lipid-specific enzymes.

2.6 Accessory Interactions and Regulation Outside the PTyr Pocket

Several SH2 proteins act as scaffolds by relying on adjacent domains to form specific and tightly regulated complexes. This phenomenon can be illustrated by the Crk protein, which contains a single SH2 domain followed by a pair of SH3 domains (nSH3 and cSH3). Its SH2 domain associates with tyrosine-phosphorylated partner proteins but is negatively regulated by intramolecular binding of the nearby pTyr221. The SH2-ligand interaction promotes the accessibility of Crk's long DE loop such that its proline-rich motif can be recognized by the Abl SH3 domain (Donaldson et al. 2002). This stable complex is flexibly oriented due the dynamic loop, giving the Abl kinase access to a range of potential phosphorylation sites in Crk and its other partners. However, another negative regulatory mechanism is mediated by the SH3 domains. The SH2 residues Arg31, Tyr104, Leu114 can also interact with the nSH3 domain's docking site for proline rich motifs, and its DE loop can bind the cSH3 domain's C-terminus (Kobashigawa et al. 2007). The latter intramolecular interaction is thus in direct competition with Abl, and is modulated by phosphorylation of the linkers.

The Src-like adaptor protein 2 (SLAP2) contains adjacent SH3 and SH2 domains which form a continuous structural unit, which contrasts with most instances where

these domains are flexibly oriented. The crystal structure reveals that a short connector closely juxtaposes the domains, which are linked by pairing of strand βA of the SH2 domain with strand E of the SH3 domain. The interface is further stabilised by hydrophobic contacts mediated by SH2 residues Tyr96, Leu99, Lys103, Leu107 and Leu110, making it structurally dependent on the SH3 domain (Wybenga-Groot and McGlade 2013). The canonical binding pockets of the two domains remain accessible on one face of the protein, suggesting that they can act in a concerted manner.

A variety of secondary interaction sites within SH2 domains are used to assemble multi-protein complexes. For example, the growth-factor receptor-bound protein 10 (Grb10) recognizes the C2 domain of the E3 ubiquitin ligase NEDD4 through it SH2 domain. The structure of the complex reveals that Grb10's pTyr pocket is not used (Huang and Szebenyi 2010). Instead, the major interface involves antiparallel pairing of βF of its SH2 domain with the β strand C of the C2 domain. This allows the SH2 domain to simultaneously interact with NEDD4 in a pTyr-independent manner on one side, while also binding a kinase domain on its other side. Such multivalent interactions highlight the fact that SH2 domains are often bifunctional, playing a scaffolding role by bringing together multiple proteins into a productive signalling complex.

Another level of complexity is demonstrated by the SH2 domains that assemble PLC-γ1 and interleukin-2 tyrosine kinase (Itk) protein complexes. The latter non-receptor PTK is recruited to the membrane by its PI-binding PH domain, whereupon its activation loop is phosphorylated by the Lck kinase. The activated Itk protein then initiates downstream lipid signalling events by phosphorylating PLC-γ1. A scaffolding role is played by PLCγ1's C-terminal SH2 domain, which docks to Itk to allow its kinase to selectively phosphorylate a critical tyrosine in the linker that precedes the SH2 domain (Min et al. 2009). PLCγ1 uses a basic patch formed by CD loop and C terminal residues within its SH2 domain to bind an acidic patch within the G helix of the Itk catalytic domain. Meanwhile the Itk SH2 domain can also dock to the Itk kinase, thus allowing it to phosphorylate a tyrosine within its own SH3 domain. However, the Itk SH2 domain uses a distinct basic patch on its surface for its engagement with the adjacent kinase. Thus the Itk kinase binds two different SH2 domains through distinct intramolecular and intermolecular docking events that leave the pTyr sites available for further signalling interactions.

In contrast, the fibroblast growth factor receptor (FGFR) kinase binds to the N-terminal SH2 domain of PLC-γ1 through yet another mechanism (JH Bae, ED Lew, 2009). The architecture of PLC-γ1 is unusual in that it contains a split PH domain containing a long loop that includes two tandem SH2 domains and an SH3 domain. The classical pTyr pocket of this nSH2 domain binds to an extended pY-L-D-L sequence in the C-terminal tail of the kinase. Moreover, it uses a secondary site involving hydrophobic and polar residues in β strand D and the BC and DE-loops to interact with the kinase helices αE and αI, β strand 8 and the loop between β7 and β8. The secondary site occupies an area similar in size to the pTyr site, and substantially strengthens the interaction, yielding a total binding affinity of 33 nM for this kinase and SH2 domain. The adjacent cSH2 domain binds a critical

phosphorylation site (pTyr-783) in the cSH2-SH3 linker of PLCγ1 (Bunney et al. 2012). The latter *cis* interaction is largely canonical, involving cSH2 Arg residues at positions 675, 694, and 696 contacting the pTyr and residues Phe706, Leu726, Leu746, and Tyr747 recognizing Val784 and Ala786 at positions +1 and +3 relative to the pTyr. Interestingly both the surfaces involved in this FGFR-SH2 complex differ completely from those observed for the complex between the Itk kinase and the C-terminal SH2 domain of PLC-γ1. Hence different interfaces are used to dock these kinase and SH2 domains, providing a basis for generating distinct complexes and signalling consequences.

The interactions between SH2 domains and kinases can play an auto-inhibitory role by limiting constitutive signalling. This is clearly seen in the Abl1 protein, for which structures of the complete protein have been resolved in the active and autoinhibited states (Hantschel et al. 2003; Nagar et al. 2006). Comparison of these two forms indicates that the SH2 domain autoinhibits the kinase by binding to the C lobe only when the N-terminal myristoyl group inserts into the base of the catalytic domain, inducing a bend in the kinase's C-terminal helix and generating a complementary surface for SH2 domain binding. In contrast, in the activated state, the SH2 domain appears to relocate 70 Å away to pack against the β sheet of the smaller N-lobe of the kinase principally via residues in the C-terminal half of helix αA including residues Ser143 and Ile145, strand βG, and the AB, CD, and BG loops. Thus the linkers between the structural domains are malleable, alternating between being sufficiently flexible to allow a range of interdomain motions, and packing within the interfaces between domains.

Phosphorylation appears to control the formation of Abl's states. A phosphorylated serine residue in Abl's N-terminal cap is bound by the His-Ser-Trp-Tyr128 sequence near the beginning of the SH2 domain and far from the pTyr site. Although this phosphorylation site is of unclear physiological significance, Tyr128 in βA of this SH2 domain is also often phosphorylated, suggesting a regulatory switch. Moreover, another residue involved in this pSer interaction site, Tyr167, is also frequently phosphorylated in cells (Hornbeck et al. 2004). Any of these three phosphorylation events would dramatically alter the interaction of the SH2 domain with the N-terminal cap of Abl. In addition, other phosphorylation switches appear to be operative in Abl signalling. The leukaemia-linked phosphorylation of Tyr139 in αA of the SH2 would block its interaction with kinase helix αE, thus compromising a key autoinhibitory interaction, as borne out by the effect of replacing this residue with an aspartate (Filippakopoulos et al. 2008). The cancer-linked phosphorylation of Tyr185 in the EF loop (Salomon et al. 2003) would interfere with SH2 ligand interactions at the +3 positions. Together this suggests that Abl is subject to control by phosphorylation at multiple SH2 interfaces. Interestingly, Abl1's phosphorylation pattern is generally also replicated in the related Abl2 protein, suggesting conserved regulatory mechanisms.

Like Abl, the Src kinase is controlled by intramolecular interaction of its SH2 and SH3 domain, and these are in turn controlled by phosphorylation sites at key interfaces. The structures of Src (Williams et al. 1997; Xu et al. 1997) and its

relative Hck (Sicheri et al. 1997) have been solved in both autoinhibited and phosphorylated states. These reveal that the SH2 and SH3 domains bind opposite the catalytic cleft and lock the kinase into an inactive conformation. The SH2 domain binds the phosphorylated C-terminal tail, although the presence of a glycine at the +3 position rather than the preferred isoleucine infers a purposefully weaker interaction. Nonetheless, residues at the −1 to +2 positions bind canonically, and complementary electrostatic interactions are found between the SH2 helix A and the kinase helix E. As a result of these autoinhibitory interactions the kinase helix C is relocated to displace the key catalytic residue Glu310 from the active site, the activation loop is rearranged to compromise substrate docking, and the N and C lobes are re-oriented. Together this prevents kinase activity through a cooperative network of individually weak regulatory interactions.

The activation of Src family kinases can be induced by dephosphorylation or loss of the C-terminal inhibitory pTyr, or by binding of ligands to the SH2 or SH3 domains. This leads to unravelling of the autoinhibited structure and decoupling of the SH3 and SH2 domains which are then available to recruit cellular partners. This can be seen in the structure of a partially activated state of dephosphorylated Src which reveals a distinct docking of the SH3 domain and linker to the N-lobe, leaving the SH2 unencumbered and free to interact with ligand proteins (Cowan-Jacob et al. 2005). Proteomics studies indicate that the Src and Hck SH2 domains are both frequently phosphorylated on a conserved tyrosine in strand E (Ballif et al. 2008; Guo et al. 2008), as is Src SH2 domain's BC loop (Hornbeck et al. 2004), these modifications would clearly affect their abilities to specifically recognize canonical SH2 ligands.

The Csk protein also consists of SH3, SH2, and kinase domains but differs from Src and Abl by the absence of conserved phosphorylation and acylation signals. The Csk structure reveals that the SH2 and SH3 domains pack on either side of the N-lobe of the activated kinase domain, assuming a variety of orientations in crystallized molecule but all involving conserved hydrophobic positions (Ogawa et al. 2002).

The Fes cytoplasmic tyrosine kinase is preceded by an SH2 and F-Bar domains. Its structure reveals the close interdomain packing of its active state, with the SH2 domain's N-terminal residues and Glu469 and Glu472 in helix A contacting the kinase helix C in the N-lobe (Filippakopoulos et al. 2008). These interactions are critical for placing the helix C correctly for catalysis in the active site, and allow the SH2 domain to feed substrates to the kinase domain by recognizing nearby pTyr-containing sequences. In this way the SH2 domain can help determine the substrate selectivity of the attached kinase.

The suppressor of cytokine signalling protein SOCS3 downregulates intracellular pathways by inhibiting the activity of Janus family kinases via its SH2 domain. Its ternary structure reveals that its SH2 domain simultaneously engages the kinase domain and a pTyr-containing peptide from the interleukin-6 receptor (Kershaw et al. 2013). The catalytic domain is bound by its conserved Gly-Gln-Met motif to SH2 residues including Tyr47 in strand A, and BC loop residues Asp72, Ser73 and Phe79 and Phe80. The linker before the SH2 domain also makes

important interactions with the kinase and precludes substrate access, emphasizing the ability of some SH2 domains to engage both receptors and kinases through canonical and accessory interactions as centrally positioned scaffolding modules.

Vav is a guanine nucleotide exchange factor (GEF) which is involved in B cell development and acts as an adaptor protein. Its SH2 domain associates with proteins including Syk, binding to the phosphorylated linker between its tandem SH2 domains. A doubly phosphorylated linker peptide binds to the Vav SH2 domain with pTyr342 and pTyr346 fitting in the canonical pTyr pocket and the specificity pocket, respectively, the latter involving a Lys at the βD3 position (Chen et al. 2013). This interaction leads to Syk phosphorylating the Vav protein on tyrosines in order to regulate its GEF activity.

Post-translational modifications other than phosphorylation can also regulate SH2 signalling assemblies. The interaction of Src with cortactin is unusual in that it can involve formation of an intermolecular disulphide bridge with the pTyr binding loop of the SH2 domain. Cortactin is a cytoskeletal protein that regulates actin-based cell motility, and is multiply phosphorylated by Src. The cross-link formed with Cys185 is transient and reversible, and may only occur during conditions of cytoplasmic oxidative stress, for example during the production of invadopodia (Evans et al. 2012). Although cysteines are present in the vicinity of the pTyr site in roughly a quarter of SH2 domains, the general utility of this alternative binding mode remain unclear.

2.7 Multivalent Interactions by Tandem SH2 Domains

Tandem pairs of SH2 domains are found in several proteins, and mediate particularly specific and tightly regulated interactions with signalling partners. The structures of zeta-chain-associated protein kinase of 70 kDa (Zap70), spleen tyrosine kinase (Syk), Shp1 and Shp2 phosphatases, PLC-γ1 and PI3 K have revealed how adjacent pairs of SH2 domains orient catalytic domains, providing insights into their intricate control mechanisms.

The Zap70 kinase mediates signalling downstream of the activated T-cell receptor, which becomes phosphorylated on a pair of tyrosine residues in its immunoreceptor tyrosine-based activation motif (ITAM). The tandem SH2 domains of Zap70 undergo a dramatic conformational change upon binding to the phosphorylated ITAM sequence. Their relative orientation changes from splayed apart to aligned for synergistic binding to an ITAM target. The structure of autoinhibited Zap70 reveals that a coiled coil forms in the linker between the SH2 domains. This docks onto the catalytic domain, and orients the two SH2 binding sites outward so as to receive pTyr ligands. Phosphorylation of a pair of tyrosine residues in the coiled linker uncouples this interface, allowing the kinase helix C to switch to the active conformation (Deindl et al. 2007).

A related mechanism is found to the Syk protein, which also binds ITAM sequences via its tandem SH2 domains. The phosphorylated ITAM peptide binds in

antiparallel orientation to the tandem SH2 domains, with the cSH2 motif interacting with the N-terminal pTyr and vice versa (Futterer et al. 1998). Their respective orientations are flexible, and a coiled coil forms in the intervening linker that mediates kinase regulatory interactions, as revealed by structures of full length Syk (Graedler et al. 2013). While the cSH2 domain remains accessible to canonical ligands in the autoinhibited state, the nSH2 would need to move significantly in order to bind a pTyr-containing sequence. Activation of the kinase involves phosphorylation of linker tyrosines, which dismantles their regulatory interface.

Structures of a PI3K complex reveal the autoinhibited states formed when the two SH2 domains of its p85β subunit bind the kinase domain in its p110β subunit (Zhang et al. 2011a; Mandelker et al. 2009). The nSH2 interferes with membrane binding by slotting its helix A between the membrane-binding C2 domain and a kinase loop that contains hotspot mutations and a Glu that projects into the pTyr pocket. A coiled coil linker fits against the kinase and connects to the cSH2 domain which locks the C-lobe of the catalytic domain into an inactive position. The pTyr site of the cSH2 domain remains exposed in the inhibited complex, which instead employs a loop between βF residue Ala674 and Tyr680 to bind the C-lobe. The cSH2 also binds to the A-Raf kinase via two separate sites that are pTyr-dependent and independent, respectively, thus increasing the potential specificity of the interaction (Fang et al. 2002). Interestingly this interface contains the two most frequently phosphorylated residues in the protein (Gu et al. 2010; Hornbeck et al. 2004). Indeed phosphorylation of serine in the PI3K SH2 domains directly prevents pTyr peptide binding, inferred a means of controlling recruitment and activation of this signalling enzyme (Lee et al. 2011).

The Shp2 protein transduces signals downstream of growth factors through the Ras-ERK1/2 pathway. The basal state of Shp2 is locked in an autoinhibited conformation by the nSH2 attaching to the catalytic site (Hof et al. 1998). In particular, the nSH2 domain inserts its DE loop into the active site of the phosphatase, engaging the catalytic cysteine and blocking substrate access. Residues in the nSH2 making contact with the catalytic domain include Asn58, Gly60, Asp61 and Ala72, and the register of αB packing changes. In contrast the cSH2 domain remains relatively free, and can orient flexibly relative to the nSH2 domain. Activation occurs upon interaction of the tandem SH2 domains with phosphorylated ligands including ITAM sequences, thus unencumbering the active site from allosteric inhibition. Germline mutations associated with Noonan and LEOPARD syndromes are clustered at this regulatory interface, and cause these dominant developmental disorders by altering the control of the phosphatase.

2.8 Multimerization of SH2 Domains

Although most SH2 domains are monomeric, the oligomeric assemblies formed by some SH2 domains can increase their ligand avidity and specificity. This phenomenon can be considered to be analogous to the interactions of tandem SH2 proteins.

For example, full-length Grb10 and its SH2 domain form dimers in solution under physiological conditions through interactions mediated by the αB helix (Stein et al. 2003). The dimer affinity is moderate at 2 μM, suggesting an equilibrium between the monomeric and dimeric states in cellular contexts, thus modulating membrane avidity of Grb10 through its adjacent phosphoinositide-binding PH domains. The similar Grb7 protein and SH2 domain also form dimers with similar affinity and interface, potentially contributing to cooperative binding to receptors, which are also dimeric when activated (Porter et al. 2007). High affinity binding of the insulin receptor by Grb14 also relies on its SH2 domain forming a homodimer mediated by αB, with Phe519 being centrally involved in the interface (Depetris et al. 2005). Thus all three relatives employ αB to mediate dimerization to facilitate receptor interactions.

In contrast, dimerization of the Grb2 SH2 domain found in crystals involves domain-swapping and compromises ligand binding affinity (Benfield et al. 2007), and hence may be considered an artefact of crystallization. An intermolecular disulphide is seen in crystals of the Src homologous and collagen-like (Shc) protein, where it stabilizes an extensive dimer interface (Rety et al. 1996). The dimer is further supported by contacts mediated by residues in the AB, CD and long BG loops, βC, and the C-terminal end of αB. Although the ligand binding pockets remain fully available, these dimers only form in solution at high protein concentrations and low pH, and hence their physiological relevance remains unclear.

The adaptor protein APS is a substrate of the insulin receptor kinase, which it binds via its SH2 domain. The APS SH2 domain was shown to form a dimer in cells. Its structure reveals a back to back dimer mediated by a long tilted αB helix that encompasses residues usually found in βE and βF (Hu et al. 2003). This dimer structure exhibits a noncanonical binding mode in which a pair of pTyr residues in the kinase's activation loop engage through the canonical pTyr site and a lysine in the βD3 position, respectively. Thus the dimer structure is responsible for the unique selectivity of this SH2 domain for doubly phosphorylated peptide ligands.

The STAT proteins derive their names from being signal transducers in the cytoplasm and activators of transcription in the nucleus. The structure of the unphosphorylated STAT protein indicates how the STAT SH2 domain binds a receptor through specific recognition of its pTyr motif (Neculai et al. 2005; Chen et al. 1998, 2003). The weak dimer interfaces which can be seen in crystals could be outcompeted by stronger SH2-mediated dimers formed upon receptor activation. The stable dimers translocate to the nucleus where the juxtaposed DNA binding domains can initiate transcription. Thus dimerization of SH2 domains directly influences the cellular destination of these signalling complexes.

3 Future Prospects

The wealth of structural information on SH2 domain-ligand complexes provides a depth of understanding into the molecular mechanisms of signal recognition. These signals are typically pTyr motifs in extended polypeptide conformations. However, other SH2 ligands are helical, turn or inverted pTyr sequences and can include unphosphorylated or serine phosphorylated peptides and even phospholipids. Hence identification of SH2 specificities within signaling proteins remains a challenge, due in part to the inherently transient and dynamic nature of these interactions, necessitating experimental validation. The multimerization surfaces and accessory protein-protein interaction sites outside the canonical SH2 pockets add further complexity, and reveal the importance of multivalency, interdomain contacts and adaptable linker elements in regulating and mediating biological interactions. These facets culminate in the subcellular localisation and activation of SH2 proteins, and must be anticipated if signalling events are to be accurately manipulated. Intelligent use of mutations and chemical probes can already be used to reprogram the biological behaviour of many SH2 proteins. As this yields quantitative insights into how pathological conditions develop and respond to intervention, SH2 domains will increasingly be seen as viable targets for the design of therapeutic agents that selectively block the progression of developmental diseases and cancers. The characterization of the various SH2-driven interactions have already revealed an astonishing diversity of ligand types and binding pockets. As this structural information is used in conjunction with phosphoproteomic and disease-linked mutation databases, more insights into SH2 signaling and deregulation will surely emerge, yielding further targets and biomarkers for mechanism-based intervention into a wider range of disease states.

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